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CONCERNING THE HYGIENIC STUDY OF ENZYME PREPARATIONS
PRODUCED BY MICROFUNGI AND THEIR POSSIBLE USE IN THE
FOOD INDUSTRY

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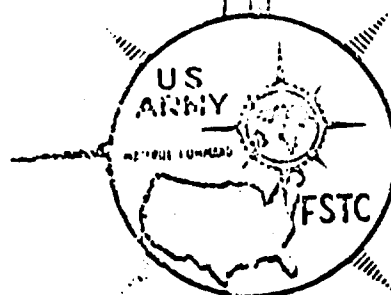
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CONCERNING THE HYGIENIC STUDY OF ENZYME PREPARATIONS PRODUCED
BY MICROFUNGI AND THEIR POSSIBLE USE IN THE FOOD INDUSTRY

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CONCERNING THE HYGIENIC STUDY OF ENZYME PREPARATIONS PRODUCED
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The wide use of physiologically active substances in varied branches of the food industry is now being proposed in connection with the increased use of chemicals in the national economy. Such active substances include not only antibiotics, vitamins, and hormones, but also enzyme preparations. The creation of a special industry for the production of enzyme preparations, as well as the development of scientific research in this field, is planned for the next few years.

Enzymes exert their own specific action, when they are present in negligible amounts. The use of enzyme preparations is extremely promising and effective, since it permits an acceleration of industrial processes and an improvement of the quality of production and gives a large economic effect.

According to the data of a number of authors [1, 2], the production and use of enzyme preparations is widespread abroad, especially in such countries as the United States, Japan, England, Czechoslovakia, the German Federated Republic, China, India, etc.

A special place among the enzymes of plant and animal origin is occupied by the enzymes that are produced by microfungi. The raw material serving as a nutrient medium for the culturing of such fungi is cheap and readily available -- most often is the waste products of the flour milling and groats industries. The producer fungus is pulverized together with the medium on special crushers and is dried while observing mild conditions of drying. The mass obtained represents the technical enzyme preparation, which serves as the starting material for the production of the purified preparation in the form of a dry powder.

The wide use of such preparations in various branches of the food industry is proposed for the near future in the Soviet Union: in the

brewing industry -- for the utilization of simple raw materials and for increasing the periods of storage of the prepared beer, in the baking industry -- for the utilization of flour with reduced bread baking properties and for increasing the yield of good quality products, in the fishing industry -- for accelerating the processes of ripening of herring during its pickling and for improving its quality, in the fruit and berry industry -- for the clarification of juices and increasing their yield, etc.

However, it is known from the literature sources that toxic forms may also be encountered among microscopic producer fungi.

Thus, according to the data of L. I. Kursanov [3], P. N. Kashkin [4], and other authors, the genus of fungi *Aspergillus* numbers more than 60 different species, among which there are also forms pathogenic for man. It is also known that the conditions of nutrition of the fungus, aeration, and temperature of the surrounding medium may sharply change not only the external appearance of the culture, but also its ability for toxin formation.

Certain fungi of the genera *Aspergillus*, *Fusarium*, *Mucor*, etc., infecting grain, kidney beans, peas, as well as feed for farm animals, may produce toxic substances under unfavorable storage conditions. The use of such products and feeds may cause illness of humans and animals [5-7].

In 1962, the first attempt was made at the Institute of Nutrition of the Academy of Medical Sciences USSR to study the effect of enzyme preparations on the animal organism.

The objects of investigation were enzyme preparations with cytolytic and amylo-proteolytic activity. The first were produced from the fungus *Trichothecium roseum*, cultured on media consisting of different coarse waste products -- oat, rye, barley, and wheat husks, the second -- from the fungi *Aspergillus oryzae* strain No. 476I and *Aspergillus awamori* (strain No. 673), cultured on a medium of wheat cuttings. In certain cases, residues of pulverized malt, malt sprouts, and yeast autolyzate were added to the basic component of the medium.

The cytolytic enzyme preparations have been proposed for use in the brewing industry, while amylo-proteolytic enzyme preparations have been proposed for use in bread baking.

The addition of cytolytic enzymes in the malting of barley promotes a breakdown of the cell walls of the endosperm, accelerates hydrolysis of the storage portions of the grain, and facilitates the access of other enzymes to them. The introduction of enzymes into the mash during the process of brewing increases the yield and quality of production, as well as its stability during storage. According to the data of R. V. Feniksova [1], L. S. Salmanova [8], S. P. Stashko [9], Ye. Ya. Kalashnikov [10], etc., preliminary industrial experiments with the addition of enzyme preparations from the fungus *Trichothecium roseum* gave positive results. The beer was distinguished by a good bouquet, transparency and stability during storage; the yield of beer was substantially increased.

The addition of enzyme preparations in the making of bread intensifies the hydrolytic processes and the intensity of fermentation of the dough, which is especially important when using flour with reduced baking qualities. Preliminary factory tests [11] also showed positive results. The bread differed from the usual bread in a more intensely

colored crust, greater porosity, and a pleasant taste and aroma. The yield of the product in this case was substantially increased.

Twenty enzyme preparations were investigated at the Institute of Nutrition of the Academy of Medical Sciences USSR. Of them, 13 were technical preparations (dried cultures of the fungus Trichothecium roseum together with the medium), five were purified preparations of "cytase" from the fungus of the same name, obtained from the All-Union Scientific Research Institute of the Nonalcoholic Beer and Wine Industry, and two were purified enzyme preparations from the fungus Aspergillus oryzae strain No. 476I and Aspergillus awamori strain No. 673, obtained from the Ukrainian Scientific Research Institute of the Food Industry. All the preparations were characterized by a definite degree of activity.

Since these preparations were proposed for use in the preparation of food products, it was necessary to determine what influence they exert upon the animal organism. For this purpose, we conducted acute and 30-day experiments on white mice, and partly on guinea pigs. According to the data of a number of authors [4-7], these animals, as well as cats and pigeons, are the most sensitive to the toxins of microfungi. In conducting the experiments, the enzyme preparations were dissolved in physiological saline, and injected into white mice (weight 20 g) directly into the esophagus with a probe, while the guinea pigs were fed from a pipette.

A total of about 1000 mice and more than 40 guinea pigs were used in this work.

Different amounts of the preparations, up to 5 g/kg of weight, do not cause death of the animals. Analogous results were obtained in acute experiments on guinea pigs.

However, in the intraperitoneal method of introduction of sterile solutions of enzyme preparations, we succeeded in obtaining the characteristic clinical picture of acute toxicosis on white mice. Sometime after introduction of the preparations, the mice became hunchbacked, listless, dishevelled. The animals refused food and drank much; their respiration became frequent and superficial. These symptoms passed after one to two days if the dose of the preparation from the fungus Aspergillus oryzae strain No. 476I did not exceed 0.75 g/kg, that from the fungus Aspergillus awamori strain No. 673 -- 2.5 g/kg, and from the fungus Trichothecium roseum -- 0.5 g/kg. When larger amounts of the preparations were administered, death of the mice with symptoms of substantial swelling of the stomach, convulsions, and disorders of motor coordination was observed.

Autopsy of the mice that died* revealed the presence of an abundant

*The autopsies of the animals of these and the subsequent experiments were performed by M. I. Razumov and B. K. Skirko.

amount of blood in the abdominal cavity (preparation from the fungus Aspergillus oryzae) or serous fluid (preparations from the fungi Aspergillus awamori and Trichothecium roseum), as a result of a disruption of the

permeability of the vascular walls of the intestines. A sharp injection of the vessels of the abdominal cavity over the entire intestine was also noted. In individual cases, signs of subacute catarrhal inflammation, with a negligible number of hemorrhages, were observed in the mucous membrane of the stomach. The mucous membrane of the small intestine was swollen over its entire extent; point and focal hemorrhages were noted in individual portions of it. In the large intestine, and especially in the rectum, the changes were weakly expressed.

In all cases, death of the mice resulted from acute peritonitis. In the case of moderate or weak manifestation of hemorrhagic diathesis, the changes in the small and large intestines were of the nature of acute or subacute enteritis.

The microscopic changes were analogous in all these cases; moreover, for a larger dose, the symptoms of hemorrhagic diathesis were more distinct, while in the case of smaller doses the picture was more smoothed out.

No pathological changes were detected in the parenchymal organs.

Thus, in an acute experiment on the intraperitoneal administration of large doses of enzyme preparations to white mice, we succeeded in obtaining a clinical and pathological picture of disease largely coinciding with the symptom complex that arises when the animals (mice, guinea pigs, cats) are fed with fusarium-infected grain [7, 5].

The experiments conducted for a period of 30 days consisted of daily peroral administration of the preparations to groups of white mice in amounts close to those proposed for use in the brewing and baking industries.

According to the technological data, in beer manufacture a purified preparation of the fungus Trichothecium roseum is added in an amount not exceeding 0.02% of the weight of the raw material to be mashed. In bread baking, a preparation of the fungus Aspergillus oryzae strain No. 476I is used in an amount not exceeding 0.02%, while a preparation of the fungus Aspergillus awamori strain No. 673 is used in an amount no greater than 0.05% of the weight of the flour used.

If we assume that these amounts are entirely retained in the finished product (which in practice is rather improbable), and that a man with an average weight of 70 kg consumes 1 liter of beer or 1 kg of bread daily, then 0.2 g of the preparation of Trichothecium roseum or Aspergillus oryzae strain No. 476I or 0.5 g of the preparation of Aspergillus awamori strain No. 673 will enter his body each time. Converted to 1 kg of weight, this comprises about 0.03 and 0.007 g, respectively.

In the experiments on mice (20 g in weight), the daily dose of the preparation, administered per os, was aggravated tenfold. Thus, during the experimental period, lasting 30 days, each mouse of the corresponding group received a dose per kg of weight of 0.9 g of the preparation of Trichothecium roseum or Aspergillus oryzae strain No. 476I or 2.1 g of the preparation of Aspergillus awamori strain No. 673.

Parallel experiments were conducted with heated preparations (20 min in a boiling water bath), since the enzyme preparations are subjected to the influence of high temperatures during the baking of the bread and the brewing of beer.

During the experiments, observations were made of the condition, behavior, and weight of the animals, as well as of their eating of food and drinking.

No deviations in the condition of the experimental animals and their weight were noted in comparison with the mice of the control group. The animals were active, ate willingly, and gained weight. After the end of the experiment, all the animals were decapitated. No pathological changes were detected in the organs and tissues of the experimental animals. Nor was any sharp difference observed between the weights of the liver and spleen in the experimental and control animals.

Since the literature contains information indicating that individual species of fungi from the genus Aspergillus cause skin diseases of the type of persistent eczemas, we conducted supplementary tests on rabbits and white mice, using the skin test method.

Solutions of a technical culture of purified preparations from the fungus Trichothecium roseum, as well as preparations of Aspergillus oryzae strain No. 476 and Aspergillus awamori strain No. 673, in the concentrations used in subchronic experiments, exerted no irritating effect when applied on the skin of the animals.

In conclusion, we should indicate that work on the study of enzymatic preparations is continuing at the Institute of Nutrition, Academy of Medical Sciences USSR.

On the basis of our work, which is only a first attempt to give a hygienic evaluation of enzyme preparations, we consider it possible to draw the following conclusions:

1. Large doses of enzyme preparations of the fungi Trichothecium roseum, Aspergillus oryzae strain No. 476I, and Aspergillus awamori strain No. 673 exerted no harmful effect upon white mice when administered perorally to animals in acute experiments.

2. In acute experiments on white mice with intraperitoneal injections of large doses, the same preparations caused a clinical and pathological picture of disease in the experimental animals, largely coinciding with the symptom complex arising when the animals are fed fusarium-infected grain.

3. Peroral administration of several aggravated "industrial" doses of enzyme preparations from the fungi indicated above to white mice over a period of 30 days caused no diseases or visible pathological anatomical changes.

4. The data obtained give no basis for objecting to the use of a preparation of the fungus Trichothecium roseum for extensive industrial testing in the brewing industry, as well as enzyme preparations of the fungi Aspergillus oryzae strain No. 476I and Aspergillus awamori strain No. 673 for the same tests in the baking industry.

5. The production of the indicated enzyme preparations and their use in the brewing and baking industries should be conducted according to the corresponding technological conditions.

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